HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

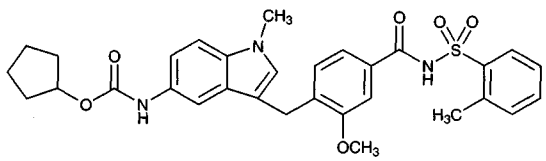
CHROMATOGRAM
Retention time: 8.15 (A), 4.37 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Zafirlukast



Molecular formula: $C_{31}H_{33}N_3O_6S$
Molecular weight: 575.69
CAS Registry No.: 107753-78-6
Merck Index: 10241

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut (LRBE) C18 SPE cartridge (Varian) with two 1 mL portions of MeOH and two 1 mL portions of water. Add 1 mL MeCN to 1 mL plasma, mix, centrifuge at 1000 g for 5 min, decant the supernatant, add it to 5 mL 10 mM pH 7.0 triethylammonium phosphate buffer. Add the diluted supernatant to the SPE cartridge, let the whole supernatant volume pass through the cartridge, elute with three separate aliquots of 500 μL MeCN:THF:triethylamine 90:10:0.2, evaporate the solvent under nitrogen at 40°, reconstitute the sample in 250 μL mobile phase, filter (0.45 μm syringe filter), inject an aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4.0 5 μm Zorbax CN
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: THF:hexane:90% glacial acetic acid 30:70:0.1
Flow rate: 0.9
Injection volume: 150
Detector: F ex 250 em 452

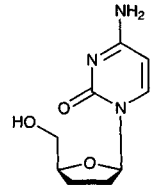
CHROMATOGRAM
Retention time: 9-10
Internal standard: 4-5(-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-a-benzylsulfonylbenzamide (ICI 198 707) (10-11)
Limit of quantitation: 750 pg/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, albuterol, aspirin, benzyl alcohol, caffeine, ibuprofen

KEY WORDS
plasma; SPE

REFERENCE
Bui,K.H.; Kennedy,C.M.; Azumaya,C.T.; Birmingham,B.K. Determination of zafirlukast, a selective leukotriene antagonist, human plasma by normal-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *696*, 131–136.

Zalcitabine



Molecular formula: $C_9H_{13}N_3O_3$
Molecular weight: 211.22
CAS Registry No.: 7481-89-2
Merck Index: 10242
Lednicer No.: 5 98

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 6 mL MeOH and 12 mL MeOH. Add 1.25 mL plasma to the SPE cartridge at 0.5 mL/min, wash with 1.5 mL water, elute with 2 mL MeOH at 0.5 mL/min. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, filter (Ultrafree-MC 0.45 μm) while centrifuging at 11900 g for 10 min, inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:water:heptafluorobutyric acid 6.5:93.4:0.1
Flow rate: 2
Injection volume: 100
Detector: UV 288

CHROMATOGRAM
Retention time: 5.8
Internal standard: 5-methyldeoxycytidine (UV 306) (3.8)
Limit of detection: 30 nM

KEY WORDS
SPE; pharmacokinetics; plasma

REFERENCE
Hawkins,M.E.; Poplack,D.G.; Pizzo,P.A.; Balis,F.M. High-performance liquid chromatographic method for the analysis of 2',3'-dideoxycytidine in human plasma, *J.Chromatogr.*, **1990**, *532*, 442–444.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Supelclean LC-18 SPE cartridge (Supelco) with 2 mL MeOH and 2 mL water. 1 mL Plasma + 5 ng IS, vortex, add to the SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH:water 20:80. Evaporate the eluate to dryness, reconstitute the residue in 50 μL MeOH:water 10:90, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:50 mM ammonium acetate 10:90
Flow rate: 1
Injection volume: 25
Detector: MS, VG Trio-3 triple quadrupole, thermospray interface with heated capillary at 280°, source 200°, repeller electrode 200 V, SIM, m/z 212 for zalcitabine, m/z 216 for IS

CHROMATOGRAM
Retention time: 8.9
Internal standard: [$^{15}N_2$,$^{2}H_2$]zalcitabine
Limit of quantitation: 0.25 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Jajoo,H.K.; Bennett,S.M.; Kornhauser,D.M. Thermospray liquid chromatographic-mass spectrometric analysis of anti-AIDS nucleosides: quantification of 2',3'-dideoxycytidine in plasma samples, *J.Chromatogr.*, **1992**, *577*, 299–304.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 400 μL ethyl acetate:MeCN 50:50, vortex for 30 s. Remove a 200 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL mobile phase, vortex for 10 s, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 60 RP-select B
Column: 125 × 4 5 μm Lichrospher 60 RP-select B
Mobile phase: MeOH:pH 7.0 phosphate buffer 5:95
Flow rate: 1
Injection volume: 10
Detector: UV 270

OTHER SUBSTANCES
Also analyzed: didanosine (UV 250)

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Mirchandani,H.L.; Chien,Y.W. Intestinal absorption of dideoxynucleosides: Characterization using a multiloop in situ technique, *J.Pharm.Sci.*, **1995**, *84*, 44–48.

SAMPLE
Matrix: blood
Sample preparation: Filter (Millipore Ultrafree-MC, 10000 molecular mass limit) 250 μL serum while centrifuging at 17000 g for 1.5 h, inject a 50 μL aliquot of the clear ultrafiltrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: Isopropanol:20 mM pH 5 sodium citrate 2.5:97.5
Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 3.0

OTHER SUBSTANCES
Extracted: didanosine, zidovudine

KEY WORDS
serum; ultrafiltrate

REFERENCE
Rosell-Rovira,M.L.; Pou-Clavé,L.; Lopez-Galera,R.; Pascual-Mostaza,C. Determination of free serum didanosine by ultrafiltration and high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 89–92.

SAMPLE
Matrix: blood, cell suspensions, perfusate
Sample preparation: Centrifuge cellular suspensions at 17000 g for 5 min, inject a 25 μL aliquot. Centrifuge perfusion fluid at 17000 g for 5 min, inject a 50 μL aliquot. Dilute 1 mL plasma with 1 mL saturated ammonium sulfate, vortex for 30 s, centrifuge at 3000 g for 2 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm Phenyl Hypersil NC-04
Mobile phase: MeOH:1.4 g/L sodium acetate 3:97, adjusted to pH 6.55
Flow rate: 1
Injection volume: 25-50
Detector: UV 271

CHROMATOGRAM
Retention time: 10
Limit of detection: 50 ng/mL

KEY WORDS
plasma

REFERENCE
Frijus-Plessen,N.; Michaelis,H.C.; Foth,H.; Kahl,G.F. Determination of 3'-azido-3'-deoxythymidine, 2',3'-dideoxycytidine, 3'-fluoro-3'-deoxythymidine and 2',3'-dideoxyinosine in biological samples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 101–107.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 100 μg/mL aqueous solution, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cyclobond I RSP (Advanced Separation Technologies)
Mobile phase: 2.5 mL/L Triethylamine in water adjusted to pH 6.5 with glacial acetic acid (Pass mobile phase through a silica column to saturate it with silica.)
Flow rate: 0.25
Injection volume: 5
Detector: UV 270

CHROMATOGRAM
Retention time: 29
Limit of detection: 0.05-0.1% (of zalcitabine)

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Scypinski,S.; Ross,A.J. Liquid chromatographic separation of zalcitabine and its stereoisomers, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1271–1276.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 5:95
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 3.89

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type anti-HIV drugs. 1. Stability studies for hairless rat skin permeation, *J.Pharm.Sci.*, **1995**, *84*, 1061–1066.

SAMPLE
Matrix: solutions
Sample preparation: Briefly vortex 100 μL of an aqueous solution with 300 μL MeCN:water:acetic acid 80:19:1, centrifuge at 15000 g for 5 min. Remove the supernatant and add it to 100 μL 1.25 mM phenacyl bromide in MeCN, heat at 80° for 45 min, evaporate to dryness under reduced pressure at room temperature, reconstitute with 60 μL water, vortex briefly, centrifuge at 15000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Prodigy ODS-2 (Phenomenex)
Mobile phase: MeCN:water:trifluoroacetic acid 16:84:0.1
Column temperature: 45
Flow rate: 2.2
Injection volume: 20
Detector: F ex 305 em 370

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: (-)-cis-5-(4-amino-1,2-dihydro-2-oxo-1-pyrimidinyl)-1,3-oxothiolane-2-methanol (3-TC)), cytidine, cytosine monophosphate, deoxycytidine
Noninterfering: guanidine, hypoxanthine, thymine, uracil, xanthine
Interfering: cytosine

KEY WORDS
derivatization

REFERENCE
Eisenberg,E.J.; Cundy,K.C. High-performance liquid chromatographic determination of cytosine-containing compounds by precolumn fluorescence derivatization with phenacyl bromide: application to antiviral nucleosides and nucleotides, *J.Chromatogr.B*, **1996**, *679*, 119–127.

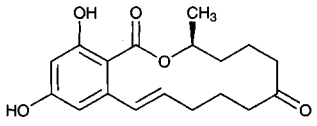
SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 5:95
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 3.89

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type Anti-HIV drugs. 2. The effect of vehicle and enhancer on skin permeation, *J.Pharm.Sci.*, **1996**, *85*, 214–219.

Zearalenone



Molecular formula: $C_{18}H_{22}O_5$
Molecular weight: 318.37
CAS Registry No.: 17924-92-4
Merck Index: 10246

SAMPLE
Matrix: solutions

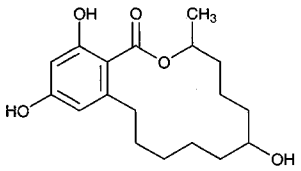
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeOH:water 80:20
Flow rate: 1
Detector: F ex 285 em 440 following post-column reaction. The column effluent mixed with 250 mM aluminum chloride hexahydrate in MeOH:water 75:25 pumped at 0.5 mL/min and the mixture flowed through a 5 m × 0.3 mm ID PTFE coil at 50° to the detector. (At the end of each day flush system with MeOH:water 75:25.)

CHROMATOGRAM
Retention time: 6.5

KEY WORDS
post-column reaction

REFERENCE
Hetmanski,M.T.; Scudamore,K.A. Detection of zearalenone in cereal extracts using high-performance liquid chromatography with post-column derivatization, *J.Chromatogr.*, **1991**, *588*, 47–52.

Zeranol



Molecular formula: $C_{18}H_{26}O_5$
Molecular weight: 322.40
CAS Registry No.: 26538-44-3, 55331-29-8
Merck Index: 10251

SAMPLE
Matrix: blood
Sample preparation: Hydrolyse serum or plasma with β-glucuronidase and sulfatase, extract with diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 40 μL buffer, add 100 μL 1.5 mg/mL dansyl chloride in acetone, shake vigorously for 30 s, heat at 100° for 5 min, inject a 20 μL aliquot. (Prepare buffer by adjusting the pH of 4 g/L sodium bicarbonate in water to 10.5 with 5 M NaOH.)

HPLC VARIABLES
Column: 250 × 2.6 PAH-10 C18 (Perkin-Elmer)
Mobile phase: Gradient. MeCN:water from 60:40 to 95:5 over 15 min (Perkin-Elmer curve 1), maintain at 95:5 for 10 min. (Flush column with MeCN at 0.1 mL/min overnight.)
Flow rate: 1
Injection volume: 20
Detector: F ex 335 em 540

CHROMATOGRAM
Retention time: 11.5
Limit of detection: 80 ng

OTHER SUBSTANCES
Extracted: diethylstilbestrol, estriol, estrone, hexestrol, zanone, zenone

KEY WORDS
derivatization; cow; sheep; plasma; serum; LOD is too high for practical detection of compounds in serum and plasma.

REFERENCE
Rhys Williams,A.T.; Winfield,S.A.; Belloli,R.C. Dns derivatization of anabolic agents with high-performance liquid chromatographic separation and fluorescence detection, *J.Chromatogr.*, **1982**, *240*, 224–229.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL dichloromethane, shake for 20 min, centrifuge at 4000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot. To measure conjugates add 1 mL residual plasma from above procedure to 1 mL pH 5.5 phosphate buffer, add 1 mL 1000 U/mL helicase (type H-2 from Helix pomatia, Sigma), heat at 37° overnight, add 10 mL dichloromethane, shake for 20 min, centrifuge at 4000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS-1 C18
Mobile phase: MeCN:MeOH:0.2% acetic acid 30:20:50

Flow rate: 1.2
Detector: radioactivity

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: taleranol, zeralanone

KEY WORDS
pig; plasma; tritium-labeled

REFERENCE
Bories,G.; Suarez,A.F. Profiling of free and conjugated [^{3}H]zeranol metabolites in pig plasma, *J.Chromatogr.*, **1989**, *489*, 191–197.

SAMPLE
Matrix: filters
Sample preparation: Sonicate filter with 2 mL MeOH for 1 h, add 1 mL water, filter (0.5 μm PTFE), inject an aliquot.

HPLC VARIABLES
Column: C18 Radial Compression (Waters)
Mobile phase: MeOH:water 60:40
Flow rate: 2
Detector: UV 236

CHROMATOGRAM
Retention time: 10.0
Limit of detection: 7 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: taleranol, zearalanone, zearalenol, zearalenone

REFERENCE
Neumeister,C.E. Environmental sampling and analysis for zeranol, *Am.Ind.Hyg.Assoc.J.*, **1987**, *48*, 919–921.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: diethylstilbestrol, trenbolone, nandrolone, dienestrol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE
Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE
Matrix: tissue

Sample preparation: Homogenize 2.5 g tissue with 10 mL acetone for 20 s, sonicate for 5 min, centrifuge at 3200 rpm. Decant the supernatant into a silanized tube. Add 8 mL acetone to the pellet and repeat the extraction. Combine the supernatants. Add to a 5 mL pipette tip containing 1.5 g alumina (80-200 mesh, Brockman activity 1) followed by an Econo-Column filled with 1.0 g AGMP-1 resin (Bio-Rad), allow to pass through by gravity. Wash with four 1 mL portions of acetone:water 95:5. Remove the alumina column, wash the ion-exchange column with 1 mL acetone:water 95:5, elute with four 1 mL portions of 10% acetic acid in acetone. Evaporate the combined eluates to dryness with nitrogen at 40°. Add 500 μL water to the residue, extract twice with 2 mL potions of ether. Combine the ether layers and evaporate them to dryness. Reconstitute the residue in mobile phase B. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelco silica
Mobile phase: Gradient. A was hexane. B was MeOH:hexane:2-propanol 45:40:15. A:B from 100:0 to 60:40 over 15 min.
Flow rate: 2.0
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 11.54
Limit of detection: 10 ng

OTHER SUBSTANCES

Extracted: diethylstilbestrol, estradiol
Simultaneous: estrone, zeralenol, zeralanone, zeralenone

KEY WORDS

chicken; muscle; normal phase; SPE

REFERENCE

Medina,M.B.; Sherman,J.T. High performance liquid chromatographic separation of anabolic oestrogens and ultraviolet detection of 17β-oestradiol, zeranol, diethylstilboestrol or zearalenone in avian muscle tissue extracts, *Food Addit.Contam.*, **1986**, *3*, 263–272.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Tekmar Model 3412-A20) 80 g ground tissue and 160 mL MeOH at medium-high speed for two 1 min intervals, centrifuge at 1500 rpm for 10 min, remove the supernatant, rinse apparatus with two 10 mL portions of MeOH. Combine the organic layers and evaporate them to 25 mL under a stream of nitrogen, adjust pH to 5.0 ± 0.2 with 20% acetic acid, add 4 mL freshly prepared β-glucuronidase solution in water (1 mg/mL, Type B-1, 500 000 U/g, Sigma), add 5 mL chloroform, mix by swirling, heat at 37° for 12-16 h, cool, add 45 mL chloroform, shake for 10 s, pass organic layer through 30-35 g anhydrous sodium sulfate (prewashed with 30 mL chloroform), extract aqueous layer with 50 mL chloroform, pass organic layer through sodium sulfate, wash sodium sulfate with two 5 mL portions of chloroform. Combine the chloroform layers and add 10 mL 2 M NaOH, shake vigorously for 30 s, discard organic layer, wash aqueous layer with two 50 mL portions of chloroform. Adjust the pH of the aqueous layer to 10.6-10.8 with 19-20 mL 1 M sodium bicarbonate solution, extract three times with 25 mL portions of chloroform, pass extracts through 30-35 g anhydrous sodium sulfate (prewashed with chloroform), wash sodium sulfate with 10 mL chloroform, evaporate chloroform layer to dryness under reduced pressure at 40°, reconstitute with 2 mL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Nova-Pak RP-C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 90 mM sodium acetate containing 10 mg/L EDTA adjusted to pH 6.9 with acetic acid.)
Flow rate: 1
Injection volume: 25
Detector: E, Bioanalytical Systems LC4B-17D, glassy carbon electrode +0.90 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 20
Limit of detection: 100 pg

OTHER SUBSTANCES
Extracted: zearalanone, β-zearalenol, zearalenone

KEY WORDS
chicken; cow; muscle; liver

REFERENCE
Roybal,J.E.; Munns,R.K.; Morris,W.J.; Hurlbut,J.A.; Shimoda,W. Determination of zeranol/zearalenone and their metabolites in edible animal tissue by liquid chromatography with electrochemical detection and confirmation by gas chromatography/mass spectrometry, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 263–271.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH: water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, wash with 1 mL MeOH, 1 mL 1 M HCl, elute with 2 mL 30 mM HCl in MeCN:MeOH 20:80. Evaporate eluate to dryness with nitrogen at 40°, take up in 100 μL MeCN:MeOH:THF:10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 22:8:13:57, inject 50 μL aliquot

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: MeCN:MeOH:THF:10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 21: 7:12:60
Flow rate: 1.5
Injection volume: 50
Detector: E, Coulochem 5100A, detector 1 0.05 V, detector 2 0.60 V

CHROMATOGRAM
Retention time: 12
Limit of detection: 1 ng/g

OTHER SUBSTANCES
Simultaneous: taleranol, zearalenol, zearalenone

KEY WORDS
muscle; liver; chicken; ox

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM

ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM
Retention time: 11.3
Limit of detection: 100 ppb

OTHER SUBSTANCES
Extracted: dexamethasone, diethylstilbestrol, medroxyprogesterone, melengestrol acetate, trenbolone, triamcinolone acetonide

KEY WORDS
cow; muscle; liver; SFE

REFERENCE
Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

SAMPLE
Matrix: urine
Sample preparation: 10 mL Urine + glucuronidase/sulfatase (Helix pomatia), incubate at 37° for 1 h, extract twice with 5 mL diethyl ether, add 225 μL water and evaporate ether under nitrogen, add 400 μL MeOH, inject a 250 μL aliquot of this mixture.

HPLC VARIABLES
Guard column: 75 × 2.1 Corasil C18
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 250
Detector: UV 240

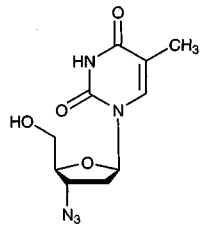
CHROMATOGRAM
Retention time: 6.5
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES
Simultaneous: 17α-methyltestosterone, 17β-trenbolone, trans-diethylstilbestrol, medroxyprogesterone, nandrolone

KEY WORDS
cow

REFERENCE
Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

Zidovudine



Molecular formula: $C_{10}H_{13}N_5O_4$
Molecular weight: 267.24
CAS Registry No.: 30516-87-1
Merck Index: 10252
Lednicer No.: 4 118

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 3 mL MeOH and 2 mL buffer, do not allow to go dry. 1 mL Plasma + 25 μL 40 μg/mL 7-ethyltheophylline in water, vortex, add to the SPE cartridge at ≤0.5 mL/min, wash with 1 mL buffer, air dry for 3 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeCN:5% acetic acid 10:90, vortex vigorously, centrifuge at 10000 g for 5 min, inject a 20 μL aliquot of the supernatant. (Prepare buffer by diluting 1.44 mL concentrated phosphoric acid to 1 L with water and adjusting the pH to 6.55 with concentrated ammonia.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. A was 1.44 mL concentrated phosphoric acid and 4 mL n-octylamine in 1 L water, pH adjusted to 6.55 with concentrated ammonia. B was MeCN. A:B from 95:5 to 70:30 over 7 min, to 20:80 over 1.5 min, return to initial conditions over 1 min, re-equilibrate for 2.5 min.
Flow rate: 1
Injection volume: 20
Detector: UV 266

CHROMATOGRAM

Retention time: 3.6
Internal standard: 7-ethyltheophylline (4.2)
Limit of detection: 7 ng/mL
Limit of quantitation: 22 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, caffeine

KEY WORDS

plasma; SPE

REFERENCE

Nadal,T.; Ortuño,J.; Pascual,J.A. Rapid and sensitive determination of zidovudine and zidovudine glucuronide in human plasma by ion-pair high-performance liquid chromatography, *J.Chromatogr.A*, **1996**, *721*, 127–137.

SAMPLE

Matrix: blood
Sample preparation: Filter (Millipore Ultrafree-MC, 10000 molecular mass limit) 250 μL serum while centrifuging at 17000 g for 1.5 h, inject a 50 μL aliquot of the clear ultrafiltrate.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: Isopropanol:20 mM pH 5 sodium citrate 2.5:97.5
Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 22.4

OTHER SUBSTANCES
Extracted: didanosine, zalcitabine

KEY WORDS
serum; ultrafiltrate

REFERENCE
Rosell-Rovira,M.L.; Pou-Clavé,L.; Lopez-Galera,R.; Pascual-Mostaza,C. Determination of free serum didanosine by ultrafiltration and high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 89–92.

SAMPLE
Matrix: intestinal mucosal homogenate
Sample preparation: Homogenate mixture + 100 μL 250 mM NaCN, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:100 mM potassium phosphate 25:75
Flow rate: 1
Detector: UV 254

KEY WORDS
rat

REFERENCE
Sinko,P.J.; Hu,P. Determining intestinal metabolism and permeability for several compounds in rats. Implications on regional bioavailability in humans, *Pharm.Res.*, **1996**, *13*, 108–113.

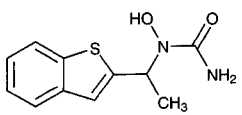
SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 20:80
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 4.18

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type Anti-HIV drugs. 2. The effect of vehicle and enhancer on skin permeation, *J.Pharm.Sci.*, **1996**, *85*, 214–219.

Zileuton



Molecular formula: $C_{11}H_{12}N_2O_2S$
Molecular weight: 236.29
CAS Registry No.: 111406-87-2
Merck Index: 10253

SAMPLE
Matrix: blood
Sample preparation: Condition a 300 μL 200 mg Analytichem Bond Elut C8 SPE cartridge with two 2 mL portions of MeOH and two 2 mL portions of water. 1 mL Plasma + 0.1-1 μg/mL IS in MeOH, mix, add to the SPE cartridge, wash with 1 mL water, dry for 3 min. Elute with two 1 ml portions of diethyl ether, evaporate under a gentle stream of air or nitrogen at 40°. Reconstitute the residue in 200 μL mobile phase or water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:MeOH:THF:water 15:5:10:70 (or 10:2:14:74 for rat blood) containing 40 mM NaH_2PO_4, 7.5 mM H_3PO_4, and 5 mM acetohydroxamic acid, pH 4.0
Flow rate: 1.0–1.5
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 15-19
Internal standard: A-66649 (Abbott Laboratories, USA) (19-25)
Limit of quantitation: 10-20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; human; monkey; rat; pharmacokinetics

REFERENCE
Granneman; G.R.; Breackman; R.A.; Erdman; K.A. Determination of a new 5-lipoxygenase inhibitor, zileuton, and its inactive *N*-dehydroxylated metabolite in plasma by high performance liquid chromatography, *Clin.Pharmacokinet.*, **1995**, *29*, 1–8.

SAMPLE
Matrix: blood
Sample preparation: Condition Bond Elut C8 cartridge with MeOH and water. Add 1 mL plasma or diluted plasma to the SPE cartridge, aspirate, wash with water, elute with ether, evaporate the eluate, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm AGP 100.4
Mobile phase: 50 mM Sodium perchlorate containing 17 mM acetic acid, adjusted to pH 2.0
Detector: F ex 260 em 321

CHROMATOGRAM
Internal standard: Abbott-66649 (Abbott Laboratories, USA)
Limit of quantitation: 10 ng/mL (R(+)), 10 ng/mL (S(-))

KEY WORDS
SPE; plasma; chiral; pharmacokinetics

REFERENCE
Wong; S.L.; Awni; W.M.; Cavanaugh; J.H.; El-Shourbagy; T.; Locke; Ch.S.; Dubé; L.M. The pharmacokinetics of zileuton 200 to 800 mg, its enantiomers, and its metabolites in normal healthy volunteers, *Clin.Pharmacokinet.*, **1995**, *29*, 9–21.

SAMPLE
Matrix: urine
Sample preparation: Directly inject a urine sample.

HPLC VARIABLES
Column: 250 × 4.6 5 μm IB-sil CN-bonded silica (Phenomenex)

Mobile phase: Isopropanol:25 mM sodium dodecyl sulfate containing 10 mM phosphate buffer 3:97, pH 3.0
Column temperature: 50
Flow rate: 1
Injection volume: 50
Detector: UV 262

CHROMATOGRAM
Retention time: 26.19
Limit of quantitation: 0.08-0.10 ppm

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Thomas,S.B.; Albazi,S.J. Simultaneous determination of the 5-lipoxygenase inhibitor "Zileuton" and its N-dehydroxylated metabolite in untreated rat urine by micellar liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 977–991.

Zinostatin

Molecular weight: ca. 16000
CAS Registry No.: 9014-02-2, 123760-07-6
Merck Index: 10302

SAMPLE
Matrix: bulk
Sample preparation: Inject 200 μL of a 660 μg/mL solution in 15 mM pH 5 sodium acetate buffer.

HPLC VARIABLES
Column: Mono Q HR 5/5 anion exchange (Pharmacia)
Mobile phase: Gradient. A was 20 mM pH 5 ammonium acetate. B was 20 mM pH 5 ammonium acetate containing 1 M NaCl. A:B 100:0 to 3:97 over 2.5 min, to 75:25 over 11 min, to 0:100 over 2 min.
Flow rate: 2
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
protect from daylight

REFERENCE
Denklau,D.; Kohnlein,W.; Luders,G.; Stellmach,J. Isolation and fast purification of neocarzinostatin by FPLC -ion exchange chromatography, *Z.Naturforsch.[C].*, **1983**, *38*, 939–942.

SAMPLE
Matrix: solutions

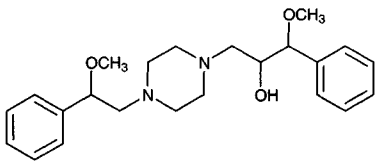
HPLC VARIABLES
Column: TSK G-3000SW (Toyo Soda)

Mobile phase: 10 mM pH 7.9 ammonium bicarbonate containing 30 mM NaCl
Flow rate: 1
Detector: UV 254, UV 280

REFERENCE

Maeda,H.; Ueda,M.; Morinaga,T.; Matsumoto,T. Conjugation of poly(styrene-co-maleic acid) derivatives to the antitumor protein neocarzinostatin: pronounced improvements in pharmacological properties, *J.Med.Chem.*, **1985**, *28*, 455–461.

Zipeprol



Molecular formula: $C_{23}H_{32}N_2O_3$
Molecular weight: 384.52
CAS Registry No.: 34758-83-3, 34758-84-4 (2.HCl)
Merck Index: 10303

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM

Retention time: 13.45

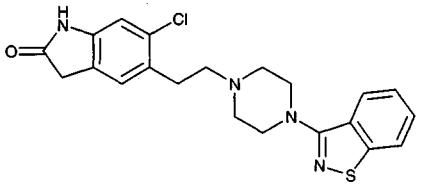
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ziprasidone



Molecular formula: $C_{21}H_{21}ClN_4OS$
Molecular weight: 412.94
CAS Registry No.: 146939-27-7, 138982-67-9 ($H_2O.HCl$)
Merck Index: 10304

SAMPLE

Matrix: blood, feces, urine
Sample preparation: Serum. 5 mL Serum + 10 mL MeCN, mix, centrifuge, wash the pellet with 2 mL MeCN, combine the supernatants, concentrate, reconstitute the residue with 200 μL mobile phase, inject an 80 μL aliquot. Urine. Condition a Sep-Pak C18 cartridge. 10 mL Urine + pH 5.0 acetate buffer, add to the SPE cartridge, wash with water, elute with MeOH, evaporate to dryness. Reconstitute the residue with 200 μL MeOH:ammonium acetate 20:80, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC basic
Mobile phase: Gradient. MeOH:20 mM pH 5.0 ammonium acetate 10:90 for 10 min, to 80:20 over 50 min, maintain at 80:20 for 7 min, return to initial conditions over 8 min, re-equilibrate for 10 min.
Injection volume: 80
Detector: UV; Radioactivity, β-RAM; MS, Perkin-Elmer Sciex API III, ion spray interface at 6000 V, collision gas argon, collision energy 25 eV

CHROMATOGRAM

Retention time: 48
Limit of quantitation: 0.5-50 ng/mL (serum)

OTHER SUBSTANCES

Extracted: metabolites

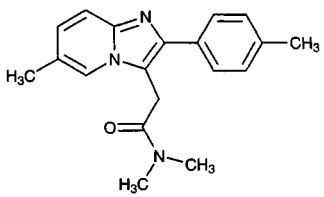
KEY WORDS

SPE; serum; pharmacokinetics; radiolabeled

REFERENCE

Prakash,C.; Kamel,A.; Gummerus,J.; Wilner,K. Metabolism and excretion of a new antipsychotic drug, ziprasidone, in humans, *Drug Metab.Dispos.*, **1997**, *25*, 863–872.

Zolpidem



Molecular formula: $C_{19}H_{21}N_3O$
Molecular weight: 307.40
CAS Registry No.: 82626-48-0, 99294-93-6 ((+)-tartrate (2:1))
Merck Index: 10321
Lednicer No.: 4 162

SAMPLE

Matrix: blood
Sample preparation: Mix 2 mL plasma, 2 mL pH 9.2 carbonate buffer, and 4 mL hexane:dichloromethane 4:3, centrifuge at 3000 rpm for 10 min. Remove the upper organic phase and evaporate it to dryness under a stream of air. Reconstitute the residue with mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 5 μm C18 Spherisorb ODS-2
Mobile phase: MeOH:THF:buffer 30:65:5 (Buffer was 10 mM potassium dihydrogen phosphate containing 0.1% triethylamine, adjusted to pH 2.6 with orthophosphoric acid.)
Flow rate: 0.8
Injection volume: 100
Detector: UV 305

CHROMATOGRAM

Retention time: 5.5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: zopiclone
Simultaneous: acepromazine, alprazolam, amisulpiride, amitriptyline, clobazam, clotiazepam, cyamemazine, desipramine, diazepam, flunitrazepam, haloperidol, imipramine, levomepromazine, lormetazepam, midazolam, nitrazepam, nortriptyline, prazepam
Interfering: bromazepam, lorazepam, niaprazine

KEY WORDS

plasma

REFERENCE

Stanke,F.; Jourdil,N.; Lauby,V.; Bessard,G. Zopiclone and zolpidem quantification in human plasma by high performance liquid chromatography with photodiode-array detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2623–2633.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM

Retention time: 11.882

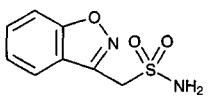
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Zonisamide



Molecular formula: $C_8H_8N_2O_3S$
Molecular weight: 212.23
CAS Registry No.: 68291-97-4
Merck Index: 10323
Lednicer No.: 4 130

SAMPLE

Matrix: blood
Sample preparation: 100 µL Plasma + 250 µL 500 mM pH 4.5 NaH_2PO_4 + 50 µL 40 mg/mL IS in MeOH + 7 mL dichloroethane, vortex for 1 min, let stand for a few min. Remove the organic layer and filter (Whatman No. 1 paper) it, evaporate the filtrate to dryness under a stream of air in a warm water bath, reconstitute the residue in 50 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.4 30-38 µm CO:Pell ODS
Column: 250 × 4.4 Hypersil 5 MOS
Mobile phase: MeCN:buffer 58:100 (Buffer was 50 mL 1 M NaOH and 58 mL 1 M acetic acid made up to 1 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 4
Internal standard: 3-sulfamoylmethyl-6-fluoro-1,2-benzisoxazole (5)
Limit of quantitation: 900 ng/mL

OTHER SUBSTANCES

Simultaneous: carbamazepine, carbamazepine 10,11-epoxide, carbamazepine 10,11-diol
Noninterfering: acetaminophen, acetazolamide, N-acetylprocainamide, ampicillin, caffeine, cefuroxime, cimetidine, chloramphenicol, clobazam, desmethylclobazam, desmethylsuximide, ethosuximide, mefenamic acid, metronidazole, naproxen, paraxanthine, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, salicylic acid, sulfamethazine, sulfamethoxazole, sulthiame, theophylline, thiopental

KEY WORDS

plasma

REFERENCE

Berry,D.J. Determination of zonisamide (3-sulphamoylmethyl-1,2-benzisoxazole) in plasma at therapeutic concentrations by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 173–181.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 600 µL allobarbital in 75 mM pH 6.8 buffer, add 200 units β-glucuronidase (Type VII-A from E. coli), incubate at 37° for 30 min, add 1 mL of the sample to an Extrelut-1 SPE cartridge, after 10 min elute with 2.5 mL MTBE, dry the eluate under a stream of nitrogen, dissolve the residue in 50 µL MeOH:water 1:1, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 25 × 4 4 µm Superspher RP-18e (Merck)
Mobile phase: MeOH:11.2 mM β-cyclodextrin in 20 mM KH_2PO_4 5:95
Flow rate: 0.8
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 7
Internal standard: allobarbital (16)
Limit of detection: 2.1 ng/mL

OTHER SUBSTANCES
Simultaneous: phenobarbital, mephobarbital

KEY WORDS
serum; SPE

REFERENCE
Eto,S.; Noda,H.; Noda,A. Simultaneous determination of antiepileptic drugs and their metabolites, including chiral compounds, via β-cyclodextrin inclusion complexes by a column-switching chromatographic technique, *J.Chromatogr.B*, **1994**, *658*, 385–390.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut SPE cartridge (cat. no. 607101) with two 1 mL portions of MeOH and two 1 mL portions of water. Add 20 μL serum, 20 μL 100 μg/mL IS in MeOH:water 50:50, and 800 μL water to the SPE cartridge, wash with 1 mL water, elute with 250 μL MeOH, inject a 40 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 17:20:63
Flow rate: 1.4
Injection volume: 40
Detector: UV 246

CHROMATOGRAM
Retention time: 3.9
Internal standard: N,N-dimethylzonisamide (8.4)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: carbamazepine, carbamazepine epoxide, phenobarbital, phenytoin
Noninterfering: valproic acid

KEY WORDS
serum; SPE

REFERENCE
Furuno,K.; Oishi,R.; Gomita,Y.; Eto,K. Simple and sensitive assay of zonisamide in human serum by high-performance liquid chromatography using a solid-phase extraction technique, *J.Chromatogr.B*, **1994**, *656*, 456–459.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 1 mL MeCN, vortex for 30 s, centrifuge at 13400 g for 2 min, inject a 20 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 75 × 4.6 TSK gel ODS-80Tm (Tosoh)
Mobile phase: MeCN:5 mM pH 4.7 KH_2PO_4 31:69
Flow rate: 1
Injection volume: 20
Detector: UV 210

KEY WORDS
serum; comparison with capillary electrophoresis

REFERENCE

Makino,K.; Goto,Y.; Sueyasu,M.; Futagami,K.; Kataoka,Y.; Oishi,R. Micellar electrokinetic capillary chromatography for therapeutic drug monitoring of zonisamide, *J.Chromatogr.B*, **1997**, *695*, 417–425.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. 200 μL Serum, plasma, or whole blood + 300 μL water + 1 mL 100 mM pH 6.0 phosphate buffer + 50 μL 100 μg/mL dimethylzonisamide in chloroform:EtOH 10:1 + 6 mL chloroform:EtOH 10:1, shake for 10 min, centrifuge at 3500 rpm for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL EtOH, centrifuge at 10000 rpm for 1 min, inject a 20 μL aliquot. Tissue. Homogenize (glass homogenizer) rat brain with 100 mM pH 6.0 phosphate buffer. 500 μL Homogenate + 50 μL 100 μg/mL dimethylzonisamide in chloroform:EtOH 10:1 + 1 mL MeCN, let stand for 30 min at room temperature, centrifuge at 14000 rpm for 1 min. Remove the supernatant and evaporate it to 200 μL, centrifuge at 14000 rpm, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Develosil ODS-7 (Chemco, Osaka)
Mobile phase: MeCN:isopropanol:1% acetic acid 11:10:70
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 285

CHROMATOGRAM

Internal standard: dimethylzonisamide
Limit of detection: 50 ng/g (brain), 100 ng/mL (blood)

KEY WORDS

rat; serum; whole blood; brain; human; plasma; pharmacokinetics

REFERENCE

Nishiguchi,K.; Ohnishi,N.; Iwakawa,S.; Yagi,J.; Nakayama,S.; Takada,S.; Nakamura,H.; Yokoyama,T.; Okumura,K. Pharmacokinetics of zonisamide; saturable distribution into human and rat erythrocytes and into rat brain, *J.Pharmacobiodyn.*, **1992**, *15*, 409–415.

SAMPLE

Matrix: urine

Sample preparation: Inject a 100 μL aliquot of urine.

HPLC VARIABLES

Column: 250 × 10 10 μm Econosil C18
Mobile phase: Gradient. MeCN:MeOH:50 mM ammonium acetate from 1.6:2.4:96 to 28:42:30 over 30 min.
Flow rate: 4
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Retention time: 21

OTHER SUBSTANCES

Extracted: metabolites

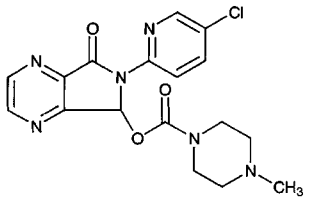
KEY WORDS

rat; preparative

REFERENCE

Stiff,D.D.; Zemaitis,M.A. Metabolism of the anticonvulsant agent zonisamide in the rat, *Drug Metab.Dispos.*, **1990**, *18*, 888–894.

Zopiclone



Molecular formula: $C_{17}H_{17}ClN_6O_3$
Molecular weight: 388.81
CAS Registry No.: 43200-80-2
Merck Index: 10324

SAMPLE

Matrix: blood
Sample preparation: Condition a 500 mg Bond Elut LRC C2 SPE cartridge with 2 mL MeOH, 2 mL MeCN:water:trifluoroacetic acid 80:20:0.1, and 5 mL Tris buffer. Mix 100 µL 100 ng/mL IS with 2 mL 50 mM Tris buffer adjusted to pH 7.5 with concentrated HCl and 500 µL plasma. Add sample to the SPE cartridge and draw through by vacuum at 2 mL/min. Wash with 20 mL Tris buffer, dry with vacuum for 1 min. Elute with 2 mL MeCN:water:trifluoroacetic acid 80:20:0.1, evaporate the eluate to dryness under a stream of nitrogen at 40°. Reconstitute the residue in 1 mL MeOH, vortex for 40 s, centrifuge at 3000 rpm for 15 min, decant the supernatant, evaporate to dryness. Reconstitute the residue in 200 µL mobile phase, vortex for 20 s. Inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 4 µm Nova-Pak phenyl Guard-Pak
Column: 300 × 4.6 5 µm C18 Spherisorb ODS-2
Mobile phase: MeOH:THF:buffer 30:65:5 (Buffer was 10 mM potassium dihydrogen phosphate containing 0.1% triethylamine, adjusted to pH 2.6 with orthophosphoric acid.)
Flow rate: 0.8
Injection volume: 100
Detector: UV 305

CHROMATOGRAM

Retention time: 4.5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: zolpidem
Simultaneous: acepromazine, alprazolam, amisulpride, amitriptyline, clobazam, clotiazepam, cyamemazine, desipramine, diazepam, flunitrazepam, haloperidol, imipramine, levomepromazine, lormetazepam, midazolam, nitrazepam, nortriptyline, prazepam
Interfering: bromazepam, lorazepam, niaprazine

KEY WORDS

plasma; SPE

REFERENCE

Stanke,F.; Jourdil,N.; Lauby,V.; Bessard,G. Zopiclone and zolpidem quantification in human plasma by high performance liquid chromatography with photodiode-array detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2623–2633.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL 2 µg/mL IS in MeCN + 1 mL 10 mM pH 8 sodium phosphate buffer + 10 mL dichloromethane:isopropanol 90:10, shake gently for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 µL mobile phase, inject a 100 µL aliquot on to a 250 × 4.6 5 µm Nucleosil silica column and elute with MeCN:MeOH 95:5 at 1 mL min, collect the fraction containing zopiclone (about 7.5 min), evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: cellulose carbamate (Société Française Chromato Colonne)

Column: 250 × 4.6 cellulose carbamate (Société Francaise Chromato Colonne)
Mobile phase: Hexane:EtOH 40:60
Column temperature: 35
Flow rate: 1
Injection volume: 100
Detector: F ex 300 em 470

CHROMATOGRAM
Retention time: 8 (-), 10 (+)
Internal standard: 6-(7-chloro-2-quinolyl)-5-hydroxy-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-7-one 4-methyl-1-piperazine carboxylate (5.5 (on achiral system)

KEY WORDS
plasma; normal phase; pharmacokinetics; chiral

REFERENCE
Fernandez,C.; Baune,B.; Gimenez,F.; Thuillier,A.; Farinotti,R. Determination of zopiclone enantiomers in plasma by liquid chromatography using a chiral cellulose carbamate column, *J.Chromatogr.*, **1991**, *572*, 195–202.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 10 μL 8.5 μg/mL IS in MeCN, let stand for 5 min, add 300 μL saturated borate buffer (pH 9.2), add 3 mL n-butyl chloride, vortex for 2 min, centrifuge at 2140 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeCN, inject a 1-30 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Spheri 5 C18
Mobile phase: MeCN:buffer 60:40 (Buffer was 1.15 g $(NH_4)H_4PO_4$ and 1 mL triethylamine in 1 L water, pH 6.8.)
Flow rate: 1
Injection volume: 10-30
Detector: UV 305

CHROMATOGRAM
Retention time: 4
Internal standard: 6-(6-chloro-2-quinolyl)-5-hydroxy-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-7-one 4-methyl-1-piperazine carboxylate (29481 R.P.) (6)
Limit of quantitation: 4 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
whole blood

REFERENCE
Boniface,P.J.; Martin,I.C.; Nolan,S.L.; Tan,S.T. Development of a method for the determination of zopiclone in whole blood, *J.Chromatogr.*, **1992**, *584*, 199–206.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma + 250 ng hydroquinidine + 1 mL pH 8 phosphate buffer + 6 mL dichloromethane, mix, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS C18

Mobile phase: MeCN:MeOH:THF:buffer 15:5:2:78 (Buffer was 10 mM trimethylamine adjusted to pH 2.5 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 305

CHROMATOGRAM
Retention time: 4.6
Internal standard: hydroquinidine (3.3)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: quinidine, quinine
Noninterfering: benzodiazepines, phenothiazines, tricyclic antidepressants, zolpidem

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Royer-Morrot,M.J.; Rambourg,M.; Jacob,I.; Bauer,P.; Royer,R.J. Determination of zopiclone in plasma using column liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1992**, *581*, 297–299.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently on a horizontal agitator for 10 min, centrifuge at 2800 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot. (Buffer was saturated ammonium chloride, diluted 25% with water, adjusted to pH 9.5 with 25% diluted ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm Nova-Pak C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 10 mM KH_2PO_4 adjusted to pH 2.6 with orthophosphoric acid. At the end of the day wash column with water at 0.8 mL/min for 1 h and MeOH at 0.8 mL/min for 1 h.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 200
Detector: UV 305

CHROMATOGRAM
Retention time: 4.05
Limit of detection: 24.8 ng/mL

OTHER SUBSTANCES
Extracted: alpidem, zolpidem, suriclone
Simultaneous: p-nitrophenol, ketotifen, tiaprofenic acid, vincristine, sultopride, pyrimethamine, nimodipine

KEY WORDS
plasma

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. High-performance liquid chromatographic assay with diode-array detection for toxicological screening of zopiclone, zolpidem, suriclone and alpidem in human plasma, *J.Chromatogr.*, **1993**, *616*, 95–103.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 100 µg/mL chlordiazepoxide hydrochloride in water + 100 µL 70 mM pH 8 phosphate buffer + 5 mL MTBE:isooctane 75:25, vortex for 30 s, cen-

trifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 75 μL hexane:EtOH 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Chiralcel OD-H
Mobile phase: Hexane:EtOH 40:60
Flow rate: 0.6
Injection volume: 50
Detector: F ex 300 em 470

CHROMATOGRAM
Retention time: 19 (-), 28 (+)
Internal standard: chlordiazepoxide (9.5)
Limit of detection: 1 ng/mL
Limit of quantitation: 2.5 ng/mL

KEY WORDS
plasma; chiral; pharmacokinetics

REFERENCE
Foster,R.T.; Caillé,G.; Ngoc,A.H.; Lemko,C.H.; Kherani,R.; Pasutto,F.M. Stereospecific high-performance liquid chromatographic assay of zopiclone in human plasma, *J.Chromatogr.B*, **1994**, *658*, 161–166.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 305

CHROMATOGRAM
Retention time: 4.05
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; almino-

profen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents

Sample preparation: 1 mL Whole blood or gastric contents + 50 µL 400 µg/mL IS in MeOH + 1 mL EtOH + 5 drops 1 M pH 9 potassium carbonate + 2 mL water + 8 mL n-hexane:MTBE 25:75, rotate for 15 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Hypersil BDS C18

Mobile phase: Gradient. A was MeCN:MeOH:1.5 M ammonium acetate:water 10:10:3:77. B was MeCN:MeOH:1.5 M ammonium acetate:water 40:40:3:17. A:B from 95:5 to 50:50 over 20 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 15.6

Internal standard: N-allylnormetazocine (Sigma) (9)

OTHER SUBSTANCES

Extracted: pentazocine

KEY WORDS

whole blood

REFERENCE

Van Bocxlaer,J.; Meyer,E.; Clauwaert,K.; Lambert,W.; Piette,M.; De Leenheer,A. Analysis of zopiclone (Imovane) in postmortem specimens by GC-MS and HPLC with diode-array detection, *J.Anal.Toxicol.*, **1996**, *20*, 52–54.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. 1 mL Whole blood + 10 µL 8.5 µg/mL IS in MeCN + 300 µL saturated pH 9 borate buffer + 3 mL n-butyl chloride, vortex for 2 min, centrifuge, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL MeCN, inject a 10-30 µL aliquot. Tissue. Stir

10 g homogenized liver tissue, 40 mL Tris buffer, and 10 mg Subtilisin-Carlsberg protease at room temperature overnight, filter through glass wool. 1 mL Homogenate + 10 μL 8.5 μg/mL IS in MeCN + 300 μL saturated pH 9 borate buffer + 3 mL n-butyl chloride, vortex for 2 min, centrifuge, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL hexane, extract with two 1 mL portions of MeCN. Combine the MeCN layers and evaporate them to dryness, reconstitute the residue in 100 μL MeCN, inject a 10-30 μL aliquot. (Prepare Tris buffer by dissolving 121 g Tris in 1 L water, adjust pH to 7.0 with concentrated sulfuric acid.)

HPLC VARIABLES

Column: 220 × 4.6 5 μm Spheri 5 silica
Mobile phase: MeCN:buffer 60:40 (Prepare buffer by dissolving 1.15 g $(NH_4)H_2PO_4$ in 1 L water and adding 1 mL triethylamine, pH 6.8.)
Flow rate: 1
Injection volume: 10-30
Detector: UV 305

CHROMATOGRAM

Internal standard: 6-(6-chloro-2-quinolyl)-7-[(4-methyl-1-piperazinyl)carbonyloxy]-6,7-dihydro [5H]pyrrolo[3,4-b]pyrazine-5-one
Limit of detection: 50 ng/g

KEY WORDS

whole blood; liver

REFERENCE

Boniface,P.J.; Russell,S.G.G. Two cases of fatal zopiclone overdose, *J.Anal.Toxicol.*, **1996**, *20*, 131–133.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with one column volume of 1 M HCl, with 2 column volumes of MeOH, and with 1 column volume of water. Hemolyze 1 mL blood with 500 μL water, centrifuge. Add 250 μL 250 ng/mL harmane hydrochloride in 200 mM NaH_2PO_4 and 1 mL serum or hemolysate or 500 μL urine to the SPE cartridge at 1 mL/min, wash with 2 column volumes of water, wash with two 500 μL aliquots of MeCN (drain completely after each wash), elute with 250 μL MeOH:35% perchloric acid 99:1 (remove the final portion of eluate by centrifuging for 20 s), inject a 10 (urine) or 25 (serum) μL aliquot of the eluate.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:0.1% tetramethylammonium perchlorate 17:83, adjusted to pH 3.8 with 10% perchloric acid
Flow rate: 1.8
Injection volume: 10-25
Detector: F ex 320 em 520, UV 305

CHROMATOGRAM

Retention time: 12.2
Internal standard: harmane (8.3)
Limit of quantitation: 2 ng/mL (serum, F), 10 ng/mL (urine, F)

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: acetaminophen, atenolol, barbiturates, benzodiazepines, chlordiazepoxide, fluoxetine, imipramine, metoprolol, nadolol, paroxetine, salicylic acid

KEY WORDS

serum; SPE

REFERENCE

Gupta,R.N. Simultaneous determination of zopiclone and its two major metabolites (N-oxide and N-desmethyl) in human biological fluids by column liquid chromatography after solid-phase extraction, *J.Liq. Chromatogr.Rel.Technol.*, **1996**, *19*, 699–709.

SAMPLE

Matrix: formulations
Sample preparation: Grind tablets to a fine powder, weigh out amount equivalent to 5 mg zopiclone, add 40 mL 100 mM HCl, sonicate for 15 min, cool to room temperature, make up to 50 mL with 100 mM HCl, filter (1.6 μm glass fiber, Whatman GF/A), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher-60 RP Select B
Mobile phase: MeCN:THF:buffer 18:1:81 (Buffer was 3.4 g monosodium hexane sulfonate and 7.0 g $NaH_2PO_4.2H_2O$ in 1 L water, pH 4.55.)
Column temperature: 25
Flow rate: 1.5
Injection volume: 20
Detector: UV 303

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 0.05% (of zopiclone)

OTHER SUBSTANCES

Simultaneous: degradation products, impurities
Noninterfering: excipients

KEY WORDS

protect from light; tablets; rugged; stability-indicating

REFERENCE

Bounine,J.P.; Tardif,B.; Beltran,P.; Mazzo,D.J. High-performance liquid chromatographic stability-indicating determination of zopiclone in tablets, *J.Chromatogr.A*, **1994**, *677*, 87–93.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 212, 300

CHROMATOGRAM

Retention time: 1.4
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 7.50 (A), 3.79 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 5-fold with 50 mM pH 8.4 phosphate buffer. 1 mL Diluted urine + 100 μL 20 μg/mL suriclone in EtOH + 10 mL dichloromethane:isopropanol 95:5, shake gently for 5 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 μL EtOH, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 11.5 min elute the fraction containing zopiclone on to column B, after 1.5 min backflush the contents of column

B on to column C with mobile phase B, elute with mobile phase B, monitor the effluent from column C. (Chiral separation of metabolites can also be achieved by collecting the fractions containing the metabolites (20.5 to 25.5 min and 46.5 to 55.5 min) on column B then chromatographing them on column C.)

HPLC VARIABLES

Column: A 150 × 4.6 5 μm Nucleosil cyanopropyl; B 60 × 4 5 μm Kromasil silica (Informatiques & Technologies); C cellulose carbamate guard column + 250 × 4.6 5 μm cellulose carbamate (Société Française Chromato Colonne)
Mobile phase: A Hexane:EtOH:MeOH 80:5:15 containing 0.18% MeOH:diethylamine 99.9:0.1 and 0.05% water; B Hexane:EtOH:MeOH:diethylamine 55:30:15:1
Column temperature: 35 (column C only)
Flow rate: A 0.7; B 1.2
Injection volume: 100
Detector: F ex 305 em 470

CHROMATOGRAM

Retention time: 20 (-), 25 (+)
Internal standard: suriclone (18 (on column A only))
Limit of quantitation: 10 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

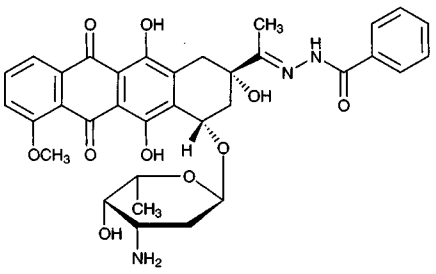
chiral; column-switching; pharmacokinetics; heart-cut

REFERENCE

Fernandez,C.; Gimenez,F.; Baune,B.; Maradeix,V.; Thuillier,A.; Farinotti,R. Determination of the enantiomers of zopiclone and its two chiral metabolites in urine using an automated coupled achiral-chiral chromatographic system, *J.Chromatogr.*, **1993**, *617*, 271–278.

Zorubicin

Molecular formula: $C_{34}H_{35}N_3O_{10}$
Molecular weight: 645.67
CAS Registry No.: 54083-22-6, 36508-71-1 (HCl)
Merck Index: 10326



SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8

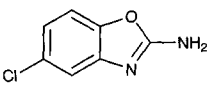
Injection volume: 50
Detector: UV 234

CHROMATOGRAM
Retention time: 8.10
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Zoxazolamine



Molecular formula: $C_7H_5ClN_2O$
Molecular weight: 168.58
CAS Registry No.: 61-80-3
Merck Index: 10328

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

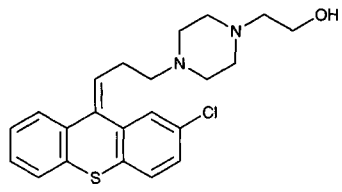
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Zuclopenthixol



Molecular formula: $C_{22}H_{25}ClN_2OS$
Molecular weight: 400.97
CAS Registry No.: 53772-83-1
Merck Index: 2455

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 25 ng IS + 300 μL EtOH + 100 μL 7 M NaOH + 8 mL hexane:isopropylamine 99.9:0.1, shake for 15 min, centrifuge at 2400 g for 5 min. Remove the organic layer and add it to 2 mL 100 mM HCl, shake for 15 min, centrifuge for 5 min. Discard the organic layer and add 200 μL 7 M NaOH and 4 mL hexane:isopropylamine 99.9:0.1 to the aqueous layer, shake for 15 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 1 mL hexane, evaporate, reconstitute with 100 μL hexane:isopropylamine 99.9:0.1, inject a 70 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb S5W silica
Mobile phase: n-Heptane:isopropanol:concentrated ammonia:water 85:15:0.4:0.2
Flow rate: 1
Injection volume: 70
Detector: UV 254

CHROMATOGRAM

Retention time: 7.5
Internal standard: 4-[3-(2-chloro-7-trifluoromethylthioxanthen-9-yl)propyl]-1-piperazineethanol (Lu 9-215) (6.5)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Extracted: trans(E)-isomer, metabolites
Simultaneous: estazolam
Noninterfering: amitriptyline, biperiden, imipramine, nortriptyline, orphenadrine, procyclidine
Interfering: benzodiazepines

KEY WORDS

serum; rat; dog; human; pharmacokinetics; normal phase

REFERENCE

Aaes-Jorgensen,T. Specific high-performance liquid chromatographic method for estimation of *cis(Z)* and *trans(E)*-isomers of clopenthixol and a N-dealkyl metabolite, *J.Chromatogr.*, **1980**, *183*, 239–245.

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Whole blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry under suction, wash with 2 mL acetone:chloroform 50:50, elute with 3 mL freshly prepared ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm Asahipak ODP-50
Mobile phase: MeCN:50 mM ammonium acetate 85:15
Flow rate: 0.6
Injection volume: 10

Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, Finnigan MAT TSP-2 interface, collision gas argon 3.0 mTorr, collision offset -17.5 V, repeller 70 V, vaporizer 130-5°, source 200°, filament off, multiplier 1500 V, dynode power 15 kV, scantime 1.20 s, MSMSC factor 0, monitor 316–271. (The effluent from the column was mixed with 50 mM ammonium acetate pumped at 0.6 mL/min. The mixture flowed to the detector.)

CHROMATOGRAM
Retention time: 3.40
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: chlorprothixene, flupenthixol, thiothixene

KEY WORDS
whole blood; SPE

REFERENCE
Verweij,A.M.A.; Hordijk,M.L.; Lipman,P.J.L. Quantitative liquid chromatography, thermospray/tandem mass spectrometric (LC/TSP/MS/MS) analysis of some tranquilizers of the thioxanthene group in whole-blood, *J.Liq.Chromatogr.*, **1994**, *17*, 4009–4110.

SAMPLE
Matrix: blood, gastric contents, tissue, urine, vitreous humor
Sample preparation: Homogenize tissue with 4 volumes water. Extract 3 mL Blood, gastric contents, urine, vitreous humor, or homogenized tissue with 1.5 mL saturated pH 9.5 ammonium chloride buffer and 5 mL chloroform:2-propanol:n-heptane 25:10:65 for 10 min. (Caution! Chloroform is a carcinogen!). Centrifuge at 3500 g for 10 min, evaporate the organic layer at 45°. Reconstitute with 30μL MeOH. Centrifuge at 10000 g for 5 min, remove 20 μL of the supernatant, inject an aliquot.

HPLC VARIABLES
Column: 150 × 2 4 μm NovaPak C18
Mobile phase: MeOH:2mM pH 3 ammonium acetate buffer 90:10
Flow rate: 0.2
Injection volume: 2
Detector: MS, PE Sciex API 100 double quadrupole, nebulizing gas nitrogen at 1.6 mL/min, curtain gas nitrogen at 1.08 mL/min, sprayer electrode +4.5 kV, electron multiplier +2.4 kV, m/z 100-450

CHROMATOGRAM
Retention time: 14.95 ((Z)-cis), ((E)-trans)

KEY WORDS
liver; kidney; lung; brain; skeletal muscle; comparison with HPLC/UV

REFERENCE
Tracqui,A.; Kintz,P.; Cirimele,V.; Berthault,F.; Mangin,P.; Ludes,B. HPLC-DAD and HPLC-MS findings in a fatality involving (Z)-cis-clopenthixol (zuclopenthixol), *J.Anal.Toxicol.*, **1997**, *21*, 314–318.

SAMPLE
Matrix: blood, gastric contents, tissue, urine, vitreous humor
Sample preparation: Homogenize tissue with 4 volumes water. 3 mL Blood, gastric contents, urine, vitreous humor, or homogenized tissue + 4 μg IS, extract with 1.5 mL saturated pH 9.5 ammonium chloride buffer and 5 mL chloroform:2-propanol:n-heptane 25:10:65 for 10 min. (Caution! Chloroform is a carcinogen!). Centrifuge at 3500 g for 10 min, evaporate the organic layer at 45°. Reconstitute with 30μL MeOH. Centrifuge at 10000 g for 5 min, remove 20 μL of the supernatant, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:THF:10 mM pH 2.6 KH_2PO_4 buffer 65:5:30

Flow rate: 0.8
Injection volume: 7
Detector: UV 228

CHROMATOGRAM
Retention time: 8.98 ((Z)-cis), 10.21 ((E)-trans)
Internal standard: prochlorperazine
Limit of detection: 7 ng/mL

KEY WORDS
liver; kidney; lung; brain; skeletal muscle; comparison with HPLC/MS

REFERENCE
Tracqui,A.; Kintz,P.; Cirimele,V.; Berthault,F.; Mangin,P.; Ludes,B. HPLC-DAD and HPLC-MS findings in a fatality involving (Z)-cis-clopenthixol (zuclopenthixol), *J.Anal.Toxicol.*, **1997**, *21*, 314–318.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 1 mL Bond Elut CN with 2 mL MeCN and 2 mL water. Add 2 mL plasma or urine to the SPE cartridge, wash with 2 mL water, elute with MeCN:n-butylamine 90:10. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb S5 CN
Mobile phase: MeCN:200 mM pH 6.5 potassium phosphate buffer:water 36:5:59 containing 6 mM dodecyl-N,N,N-trimethylammonium bromide
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: F ex 260 em 435 following post-column photolysis with a low-pressure 8 W mercury UV light in a 5 m × 0.5 mm i.d.coil of PTFE tubing

CHROMATOGRAM
Retention time: 9
Limit of detection: 0.05 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
protect from light; plasma; SPE; post-column photochemical derivatization

REFERENCE
Hansen,B.B.; Hansen,S.H. Determination of zuclopenthixol and its main N-dealkylated metabolite in biological fluids using high-performance liquid chromatography with post-column photochemical derivatization and fluorescence detection, *J.Chromatogr.B*, **1994**, *658*, 319–325.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 16.325

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Inject 1 mL onto column A. Elute column A onto column B with mobile phase for 30 s then remove it from the circuit. Elute column B with mobile phase and monitor the effluent.

HPLC VARIABLES
Column: A 10 × 6 packed with 40 μm material from a Bond Elut cartridge (cat. no. 620303); B 100 × 4 3 μm Spherisorb ODS Superpac
Mobile phase: MeCN:85% phosphoric acid:triethylamine:water 49.55:0.225:0.225:50
Flow rate: 0.65
Injection volume: 1000
Detector: UV 238

CHROMATOGRAM
Retention time: 3.30

OTHER SUBSTANCES
Simultaneous: alprazolam, amitriptyline, chlorpromazine, chlorprothixene, clomipramine, desclomipramine, desmethylimipramine, diazepam, flunitrazepam, imipramine, levomepromazine, maprotiline, nortriptyline, promethazine, thioridazine, thioridazine sulfone, thioridazine sulfoxide, trimipramine, zimeldine
Noninterfering: carbamazepine, clonazepam, lorazepam, nitrazepam, oxazepam, phenytoin
Interfering: fluphenazine, haloperidol, perphenazine, protriptyline

KEY WORDS
column-switching

REFERENCE
Svensson,C.; Nyberg,G.; Mårtensson,E. High-performance liquid chromatographic quantitation of amitriptyline and nortriptyline in dialysate from plasma or serum using on-line solid-phase extraction, *J.Chromatogr.*, **1988**, *432*, 363–369.